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Isolation of Probiotic Bacteria from Fermented White Rice Gruel

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Abstract

The present study focused on isolating probiotic bacteria from the fermented cooked white rice gruel. Probiotics are live microorganisms that offer various health benefits. Fermented rice has long been recognized for its probiotic properties. For this study, rice was fermented naturally without the addition of any starter culture to mimic traditional practices. The nutrient rich composition of rice combined with the fermentation process facilitates the proliferation of beneficial bacteria. The bacterial isolation was performed using MRS agar plates. The phenotypic characteristics including morphological traits (Gram staining, colony size, shape, and colour) and biochemical properties (Catalase, Indole, Methyl Red, Voges- Proskauer, Citrate, and carbohydrate fermentation tests) were analyzed and species of the isolated bacteria was identified through 16S rRNA sequencing. Three species of lactic acid bacteria were identified, a *Lactiplantibacillus plantarum, Weissella confusa,* and *Leuconostoc mesenteroides*.

Keywords: Fermented rice, Probiotics, *Lactiplantibacillus plantarum, Weissella confusa, Leuconostoc mesenteroides*

1. Introduction

Fermented foods and beverages plays a crucial role in human diet. Fermented foods contain probiotics like *Lactobacillus*, *Lactic acid bacteria* etc. Fermentation can enhance nutrition and there by fermented food can improve the health and control disease (Borresen *et al.,* 2012). Fermentation not only enhances the nutritional value of food but also increases its bioavailability, making essential nutrients more accessible to the body. Rice based fermented foods are popular in various countries, where they are prepared using natural fermentation process with a mixed culture of microorganisms. This spontaneous fermentation results in the development of distinct flavours and textures.

Fermentation process removes extra fat from the rice and it becomes enriched with vital micronutrients such as vitamin K, B complex, calcium, iron, magnesium, potassium and selenium. The mild sweet and sour taste of fermented rice is primarily attributed to the production of lactic acid by lactic acid bacteria (Lu. *et al.,* 2008). In certain preparations, ingredients like salt, yogurt, spices and leafy vegetables are added to enhance the flavour and nutritional value (Ray *et al*., 2016). Fermentation significantly improves the bioavailability of essential minerals by enzymatically reducing phytate content, which is a known inhibitor of mineral absorption (Kumar *et al.,* 2012).

Fermented foods are rich source of probiotics. Probiotics are live microorganisms they are intended to have health benefits when consumed in adequate amount. Probiotics have been defined as “a live

microbial adjuvants that positively influence the host's microbial community, improving nutrient utilization and enhancing the host's response to diseases" (Graciela, F. V. *et al.,* 2001). These microorganism exhibit remarkable adaptability and survivability, making them effective in various applications including gut health management, immune modulation and disease prevention. While probiotics have been isolated from diverse sources, studies focusing on fermented rice as a potential reservoir of probiotic strains remain limited. Fermented rice represents a promising avenue for isolating beneficial microorganisms, given its widespread consumption and traditional preparation methods in many culture.

In this study, the white rice variety "Ponni" was chosen for its popularity and nutritional value. The primary objective of this research was to isolate and identify probiotic bacteria from fermented white rice at the species level using 16S rRNA sequencing. The aim of the present study is to evaluate the phenotypic and biochemical characteristics of the isolated bacterial strains to understand their probiotic potential and lay the groundwork for future investigations into their health benefits.

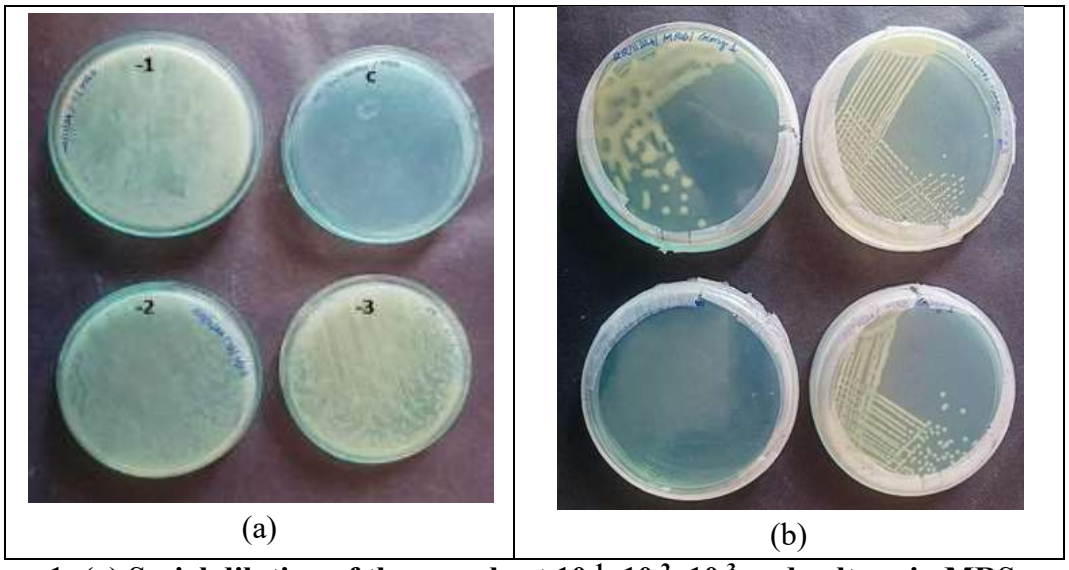
2. Materials and Methods

2.1 Preparation of Rice Sample

A total of hundred grams (100g) of white rice was thoroughly cleaned to eliminate any contaminants and cooked in water until a soft texture was achieved. The cooked rice was immersed in distilled water and left to ferment overnight in a clay pot under room temperature (Jeyagowri *et al.,* 2023).

2.2 Serial dilution and Spread plating

The first step in the isolation of probiotic bacteria is to maintain the sample in adequate conditions before incubation in selective media. 10 ml of fermented white rice samples were homogenized quickly and serially diluted to 10^{-1}, 10^{-2} and 10^{-3} and were cultured in MRS (de Man, Rogosa, and Sharpe) media (Figure 1). The culture plates were maintained at 37°C for 24 hours under anaerobic conditions. The colonies obtained were isolated and sub-cultured by streaking on a new MRS agar plate (dos Santos Leandro *et al.,* 2021).



(a) (b)

Figure 1: (a) Serial dilution of the sample at 10^{-1}, 10^{-2}, 10^{-3} and culture in MRS media; (b) Morphologically distinct three colonies subculture in MRS media

2.3 Morphological and Biochemical Characterization

The bacterial isolates were characterized using colony morphology, gram staining, and biochemical tests. Biochemical tests include the Catalase test, IMViC test and carbohydrate fermentation test.

2.3.1 Catalase Test

The catalase test was done by dropping 3% hydrogen peroxide solution into 1 ml of culture broth on the microscopic slide (Bisen and Verma, 1998).

2.3.2 IMViC Test

The Indole, Methyl Red, Voges- Proskauer, Citrate test is a series of four biochemical tests used to identify and characterize microorganisms including probiotics. The IMViC test is commonly used to identify Lactobacilli and Bifidobacteria, which are common probiotic genera (Chowdury *et al.,* 2012; Wang *et al*., 2020).

2.3.3 Carbohydrate Fermentation

The test was performed to evaluate the ability of probiotics to ferment various carbohydrates and produce metabolites such as short-chain fatty acids, lactic acid and acetic acid. The carbohydrate fermentation broth was prepared with the desired sugars (glucose, maltose, sucrose and starch) and phenol red. The broth was inoculated with bacterial culture using a sterile loop. Durham's tube was then inserted in an inverted position in the test tube and the tubes were incubated at 37°C for 24-48 hours.

2.4 Molecular Identification of Bacteria by 16S rRNA Sequencing

2.4.1 DNA Isolation and PCR Analysis

A part of the culture is taken in a microcentrifuge tube and 25µl of proteinase K were added and incubated at 56°C in a water bath. DNA isolation and PCR analysis was done according to Makut *et al.*, (2022). The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v. 5.1 (Drummond *et al.,* 2010).

2.4.2 Sequence alignment and Phylogenetic analysis

The PCR products were subjected to sequencing by using FASTA. The sequences were blasted in GenBank with BLASTn. The phylogenetic tree was constructed using MEGA 11 software (Dowarah *et al.,* 2018; Makut *et al.,* 2022).

3. Result

3.1 Morphological and Biochemical Characteristics of Isolates

Morphologically distinct three colonies were isolated from MRS (de Man, Rogosa, and Sharpe) culture based on their size, shape, and colour (Fig 1). First and second colony appears to be gram-positive rods and the third colony appears gram positive coccus (Fig.2). Morphological and biochemical identification of the bacterial isolates from fermented white rice is shown in Table 1.

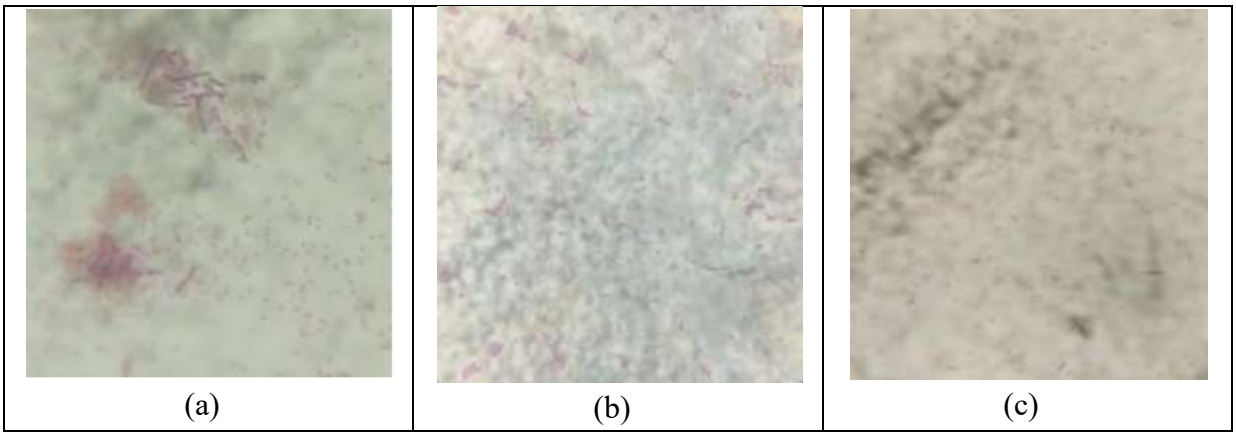


Figure 2: (a) Gram staining of first colony; (b) Gram staining of second colony; (c) gram staining of third colony

Table 1: Morphological and Biochemical test for bacterial isolates from fermented white rice

Morphological and Biochemical Identification	Colony 1	Colony 2	Colony 3
Size	L	M	S
Shape	R	R	R
Color	W	CW	PY
Gram staining	+	+	+
Catalase	-	-	-
MR	+	+	+
VP	-	-	+
Citrate	-	-	-
Glucose	+	+	+
Maltose	+	+	+
Sucrose	+	+	+
Starch	+	+	+

L- Large; M- Medium; S- Small; R- Round; W- White; CW- Creamy white; PY- Pale yellow; + Positive; - Negative

3.2 Molecular Identification of Bacterial Isolates

Genomic identification was conducted by amplifying and sequencing of nucleotide region. The nucleotide sequences of the isolates were analyzed and matched with entries in the GenBank database. The isolated bacteria are identified up to the species level (Table 2).

Table 2: Identification of Isolated Bacteria from Fermented White Rice using 16S rRNA sequencing

Colony	Isolated Bacteria	Identity
1	*Lactiplantibacillus plantarum*	93.82%
2	*Weissella confusa*	99.40%

3	*Leuconostoc mesenteroides*	95.99%

4. Discussion

Fermented foods are staple in traditional diet have been recognized for their health promoting properties due to the presence of probiotics. Diverse microbial communities include various strains of Lactic Acid Bacteria (LAB) such as *Lactobacillus plantarum, L. paraplantarum, Lactococcus lactis* etc. have been isolated from various Indian fermented foods (Angmo *et al.,* 2016). A recent study highlights the isolation of potentially probiotic bacteria such as *Pediococcus pentosaceus* and *lactobacillus plantarum* from fermented rice gruel prepared using Ponni variety of rice (Kowsalya *et al*., 2022). The *Lactiplantibacillus plantarum* is well documented for its resilience in gastrointestinal conditions, adherence to intestinal epithelial cells and antimicrobial properties (Garcia-Gonzalez *et al.,* 2021). Its metabolic versatility also enhances the nutritional value of fermented foods by synthesizing bioactive compounds including vitamins and organic acids (Yilmaz *et al.,* 2022). *Lactobacillus plantarum* UBLP40 was isolated as potential probiotic from traditional indigenous fermented foods (Ahire *et al.,* 2021).

The bacterium *Weissella confusa* is recognized because of their probiotic potential and ability to survive in gastrointestinal tract and produce antimicrobial exopolysaccharides (Teixeira, *et al.,* 2021). It has been associated with potential health benefits such as strengthening the immune system and maintaining gut microbiota balance (Thant *et al.,* 2024). *Weissella confusa* strain GCC 19R1 was isolated from traditional fermented sour rice in Cachar district of Assam, India (Nath *et al.,* 2021). In a separate study, *Weissella confusa* isolated from traditional Indian fermented food such as idli batter and noted its robust growth and survival under conditions simulating the gastrointestinal environment (Sharma *et al.,* 2018). The bacterium *Leuconostoc mesenteroides* plays a crucial role in the fermentation process by producing lactic acid and exopolysaccharides (Su *et al.,* 2024).

The use of 16S rRNA sequencing in this study provided a reliable and precise method for identifying the isolated strains conforming their probiotic potential. The findings align with earlier reports on the presence of diverse LAB strains in fermented foods and highlight the role of traditional fermentation practices in enriching probiotic diversity. The metabolic activities of these bacteria during fermentation often result in the synthesis of bioactive compounds such as organic acids, bacteriocins and vitamins which further augment the nutritional profile of these foods. The diversity of microbial strains in fermented foods is influenced by several factors, including the raw materials used, environmental conditions during fermentation and traditional preparation methods.

5. Conclusion

The focus on fermented foods as a reservoir of beneficial probiotics microorganisms has gained significant momentum in recent years. The current study underscores the potential of fermented white rice gruel as a rich source of probiotics, with isolates identified as *Lactiplantibacillus plantarum, Weissella confusa* and *Leuconostoc mesenterodes*. This discovery highlights the significant microbial diversity in rice based fermented foods, which has long been a part of traditional diets. Fermented white rice gruel being a low cost and easily accessible food, could serve as a natural probiotic source, particularly in regions where commercial probiotic have limited access. Future research should focus on characterizing the functional properties of these isolates such as their ability to survive in simulated gastrointestinal conditions, adherence to gut epithelium and specific health benefits.

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